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Review

Stress-Related Alterations of Visceral Sensation: Animal Models for Irritable Bowel Syndrome Study

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Stressors of different psychological, physical or immune origin play a critical role in the pathophysiology of irritable bowel syndrome participating in symptoms onset, clinical presentation as well as treatment outcome. Experimental stress models applying a variety of acute and chronic exteroceptive or interoceptive stressors have been developed to target different periods throughout the lifespan of animals to assess the vulnerability, the trigger and perpetuating factors determining stress influence on visceral sensitivity and interactions within the brain-gut axis. Recent evidence points towards adequate construct and face validity of experimental models developed with respect to animals' age, sex, strain differences and specific methodological aspects such as non-invasive monitoring of visceromotor response to colorectal distension as being essential in successful identification and evaluation of novel therapeutic targets aimed at reducing stress-related alterations in visceral sensitivity. Underlying mechanisms of stress-induced modulation of visceral pain involve a combination of peripheral, spinal and supraspinal sensitization based on the nature of the stressors and dysregulation of descending pathways that modulate nociceptive transmission or stress-related analgesic response.
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Key Words
Irritable bowel syndrome; Models, animal; Pain

Introduction

Alterations of visceral sensation such as enhanced perception of physiological or experimental visceral stimuli along with hypervigilance to those, are at the origin of visceral hypersensitivity, a phenomenon commonly considered to play a major role in the pathophysiology of irritable bowel syndrome (IBS).[1-7] Epidemiological studies have implicated stress of psychosocial, physical or immune origin as a trigger of first onset or exacerbation of IBS symptoms.[8-10] Early adverse life events in the form of emotional, sexual, or physical abuse are major predisposing factors for the

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development of IBS later in life.[11,12] Childhood trauma, especially in genetically predisposed individuals, is thought to induce persistent changes in the brain arousal response system that impacts on the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis.[12] In adult IBS patients, acute stress episodes, chronic social stress, anxiety disorders, and maladaptive coping style determine the illness experience, health care-seeking behavior as well as treatment outcome.[12,13] Stress-related psychosocial factors such as somatization, neuroticism, and hypochondriasis are also important predictors in the development of post-infectious IBS.[14,15] Emotional or physical stressors may cause disturbances at every levels of the brain-gut axis including the central, autonomic and enteric nervous systems and affect regulation of visceral perception and emotional response to visceral events.[16]

Over the past 15 years, various animal models have been developed to get insight into the underlying mechanisms of visceral hypersensitivity and the influence of stress on visceral pain pathways.[1,17-20] While in humans the evaluation of visceral sensitivity is predominantly based on the conscious perception to gut distension, the measurement of this subjective response cannot be performed in animal studies. Objective evaluation of responses to visceral stimulation in clinical studies includes the assessment of reflex activity (eg, a somatic nociceptive cutaneo-muscular flexion reflex can be inhibited by painful visceral stimulation) or evoked central processes (eg, changes in activation of the anterior cingulated cortex involved in pain inhibition).[21,22] Indeed, during the last decade functional imaging techniques have been applied successfully to examine the human brain response to noxious visceral stimuli.[23] In experimental animals, the pattern of brain and spinal circuitries activated by various stressors and colorectal distension (CRD) under basal or hypersensitive state have been early on mapped in a number of studies using the induction of the Fos protein expression as a direct marker of neuronal cell activation and double immunohistochemical labeling to identify the phenotype of Fos positive spinal and supraspinal neurons.[24-31] Recently, preliminary reports applied imaging techniques to get insight into brain circuit activated by visceral stimulation in rodents. Similarity in some regional brain activation induced by CRD have been found when comparing Fos expression and functional magnetic resonance imaging.[32] In addition this comparative study indicates that both methods are complementary as Fos immunohistochemistry provides a higher spacial resolution over imaging while imaging displays a higher sensitivity to detect a large number of brain area. Development of imaging in conscious animals with removal of additional stress linked with conditions of functional imaging monitoring will enable bridging the gap between the multidimensional nature of human pain experience and preclinical studies.[33]

In this review we will outline some of the most relevant preclinical models that have been developed, comment on their contribution to our understanding of stress modulation of visceral pain mechanisms, and assess the clinical relevance of these preclinical studies to unravel potential molecular targets to alleviate visceral pain symptoms in IBS.

Stress Pathways: Corticotropin Releasing Factor Signaling as an End Point Effector

First coined by the endocrinologist Hans Selye, the term "stress" defines the physiological adaptive responses to real or perceived emotional or physical threats ("stressors") to the organism homeostasis.[34] When exposed to an acute threatening challenge, the body engages a "fight or flight" response[35] driven by sympathetic activation leading to rapid heart rate and respiration, increased arousal, alertness, and inhibition of acutely non adaptive vegetative functions (feeding, digestion, growth and reproduction).[34] Concurrently, a negative feedback is activated to terminate the stress response and bring the body back to a state of homeostasis or eustasis,[36] that engages neural, neuroendocrine and immune components, a process called allostasis[37] or "stability through changes".[37,38] However, persistence or chronicity of the stressors can overload this adaptive system which then becomes defective or excessive. The organism is no longer brought back to basal homeostasis leading to a state of allostatic load[37,39] or "cacostasis."[36] This state lies at the origin of a variety of stress-related diseases that develop in the context of a vulnerable genetic, epigenetic and/or constitutional background.[36] The pathogenesis of stress-induced disorders affects the whole body, including the viscera of which the gastrointestinal (GI) tract is a sensitive target.[36,40]

Over the past decades, important components of the stress-activated pathways whereby the brain translates stimuli into final integrated bodily response have been identified through the characterization of corticotropin releasing factor (CRF) signaling system. This is composed of the 41 amino acid peptide CRF, and related peptides, urocortin 1, urocortin 2 and urocortin 3 along with the CRF receptors CRF_1 and CRF_2 and their variants which display specific affinity for CRF and related agonists.[41] The development of selective CRF receptor antago-

nists has also largely contributed to delineate the role of activation of CRF receptor subtypes in the stress response.[42,43] In particular convergent reports indicate that the activation of CRF_1 receptor underlies the multiple faceted components of the stress response.[40,44,45] CRF/CRF_1 signaling plays a primary neuroendocrine role in stimulating the HPA axis leading to the release of adrenocorticotropic hormone and corticosterone in rodents and cortisol in humans.[43,46] In addition the CRF signaling system also acts as a neurotransmitter/neuromodulator to coordinate the behavioral, immune, and visceral efferent limbs of the stress response.[44,45,47-49] It does so via the activation of the locus coeruleus and its noradrenergic projections to the forebrain which contribute to arousal, alertness as well as the modulation of forebrain, hindbrain and spinal sites regulating the autonomic nervous system activity leading to the stimulation of the sympathetic nervous system and release of catecholamines,[50-52] and sacral parasympathetic activity while decreasing vagal efferent output[53-55] that influences immune and visceral function.[56,57] In addition the brain CRF/CRF_1 signaling pathway is involved in stress-related induction of anxiety/depression[44,45,58] and alterations of colonic motor and visceral pain while both central and peripheral CRF_2 receptor activation may exert a counteracting influence.[59-63] Moreover recent experimental and clinical studies point to an equally important contribution of the peripheral CRF/CRF_1 signaling locally expressed in the gut to the GI stress response.[19,64]

Visceral Pain Pathways

Pain perception in peripheral tissues depends on the signal transmission from the site of pain origin to the CNS. Nociceptors (receptors activated by noxious stimuli)[65] located in 2 sets of primary small afferent fibers (C and Aδ afferents) innervating the viscera that project to distinct regions in the CNS,[66] are the primary pathways of pain transmission. From the esophagus to the transverse colon, the GI tract innervation is provided by vagal afferent fibers originating in the nodose ganglia and projecting centrally to the nucleus of the solitary tract. Pelvic nerve afferent fibers, which originate in the lumbosacral dorsal root ganglia, and project centrally to the lumbar 6 - sacral segments of the spinal cord innervate the remaining part of the large bowel (descending and sigmoid colon, rectum). The entire GI tract is also innervated by afferent fibers contained in the splanchnic nerves projecting to the thoracic 5 - lumbar 2 segments of the spinal cord.[67] Even though visceral afferents constitute only 10% of all afferents, they are able to monitor changes in the gut milieu and participate in the transmission of visceral sensory information.[68,69] Of note, vagal afferents do not encode painful stimuli however, changes in their activity can modulate nociceptive processing in the spinal cord and the brain.[68,70,71]

Upon entering the dorsal horn, visceral primary afferents carried out by the pelvic and splanchnic nerves terminate in spinal cord laminae I, II, V and X[72] converges onto spinal neurons in the lumbosacral segments and thoracolumbar segments respectively. Lumbosacral segments process reflex responses to acute visceral pain, while thoracolumbar segments' involvement in normal visceral sensation is uncertain,[73] however, both segments process inflammatory stimuli.[73] Subpopulations of neurons within the dorsal horn project to discrete nuclei within the thalamus (ie, ventral posterior lateral thalamus) as well as other structures in the brain stem (parabrachial nucleus, periaqueductal gray, nucleus tractus solitarius). From the thalamus, the information is conveyed to cortical areas involved in sensory processing (such as the somatosensory cortex) or those involved in processing emotional or affective information (such as the anterior cingulate gyrus and insular cortex).[65,74]

In addition to the ascending system, which enables pain perception described above, other neural circuits originating from supraspinal sites can influence nociceptive activity in the spinal cord and in primary afferents, a system referred to as descending pathways.[75] There are 2 types of descending control pathways: inhibitory, which produce analgesia (periaqueductal gray, locus coeruleus) and facilitatory which produce hyperalgesia (rostroventral medulla and OFF and ON cells).[76,77]

Visceral Pain Monitoring in Rodents

The primary readout and the standard assay for the measurement of visceral pain in rodents consists in the monitoring of abdominal muscles contraction or visceromotor response (VMR) to controlled isobaric distensions of the distal colon by an inflatable balloon.[78] The VMR can directly be assessed as electromyographic (EMG) signals monitored via surgically-implanted recording electrodes in external or internal abdominal muscle which are either externalized through the skin (abdomen, neck)[79-81] or connected to radiotelemetric implants in the abdominal cavity.[82,83] Although the method is of significant value in the field of visceral pain study, it has experimental shortfalls such as damage to EMG electrodes, loss of signal and electrical interferences which is of particular concerns in chronic experimental settings. Additionally, EMG surgery involves skin and/or mus-

cle incision depending on the technique used (subcutaneous abdominal electrodes or intraperitoneal cannula) and chronic implantation of a foreign body. Even though no data are available in the literature in relation to the impact of chronic EMG electrodes placed into the abdominal wall, such intervention could induce a host-tissue response with local micro-inflammation (neutrophils, lymphocytes and macrophages) as it has been shown for other types of implants in the skin and peritoneum.[84,85] A recent report suggests that the preconditions of animals (EMG surgery, and post-surgical delivery of antibiotic and single housing) has considerable impact on their visceral pain responses, particularly in the context of stress studies.[86] Other approaches consist of recording manometric changes in the pressure of the balloon inserted into the distal colon[86,87] or changes in pressure inside the colonic lumen.[19,88] These 2 later techniques present the advantage of being minimally invasive as they do not require surgery and

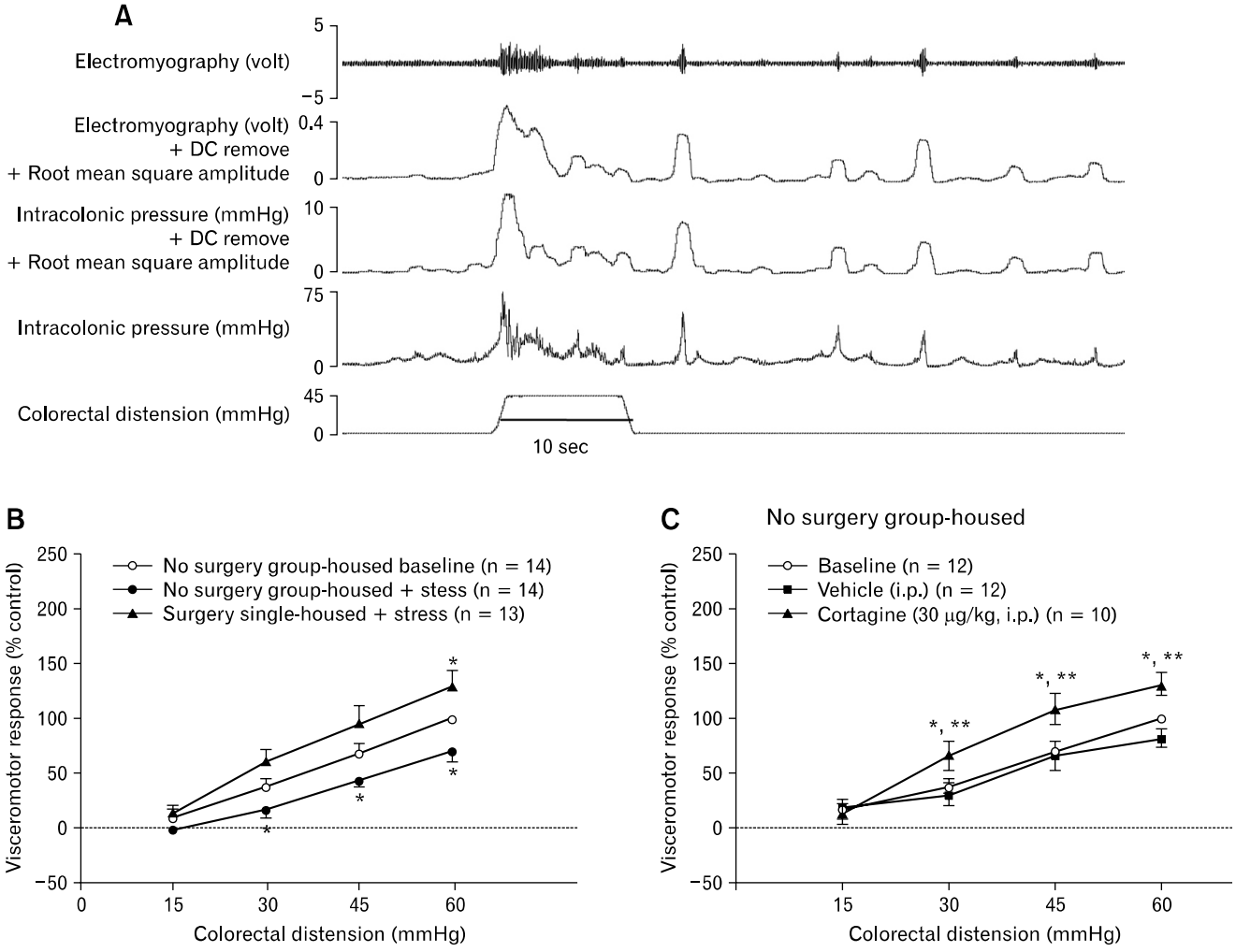

Figure 1. Differential influence of intermittent repeated stress on visceral response to colorectal distension (CRD) in rodents with or without surgical procedure for recording visceral pain (Adapted from Larauche et al[19,88]). (A) Original and rectified representative electromyographic (EMG) and intraluminal colonic pressure (ICP) traces recorded simultaneously on the same mouse in response to CRD (45 mmHg, 10 seconds). When both raw EMG (upper line) and ICP (second line to the bottom) signals are analyzed in Spike 2 by computing "DC Remove" 1 second to exclude all slow events > 2 seconds (ie, colonic smooth muscle contractions) and "root mean square amplitude" to extract the area under the curve of the signal, the resulting EMG and phasic ICP signals (middle lines) present a significant overlap. (B) Mice were equipped with EMG electrodes or not and exposed to water avoidance stress for 1 hour per day for 10 days tested with ICP for visceromotor response (VMR) to CRD. (C) Intraperitoneal injection of the selective corticotropin releasing factor receptor subtype 1 agonist, cortagine-induced visceral hypersensitivity in C57BL/6 mice tested with ICP for VMR to CRD. Data are expressed as mean ± SEM, n = 10-14 per group as specified in graph legends. $^{*}P < 0.05$ compared with baseline, $^{**}P < 0.05$ vs vehicle.

post-surgical treatments such as antibiotic, analgesics which can affect the visceral pain responses and still remain an objective and sensitive measure of abdominal contractions (Fig. 1). However, they require the animals to be partially restrained in Bollman cages, a context to which they need to be habituated and which by itself may bring a component of stress.

Behavioral approaches such as operant behavioral assays[78] have also been used in early studies and capitalized on the learning and fear behaviors of animals in response to painful CRD. Visual monitoring of the abdominal withdrawal reflex[89] has also been applied in a few studies, and while having the great advantage of being one of the less invasive technique employed to date, it is a very subjective method. Indirect endpoints such as Fos or extracellular signal-regulated protein kinase induction in the CNS,[29,62,90-92] and functional brain imaging of integrated brain responses to nociceptive stimuli[33,93] have also been utilized in some studies. These approaches allow for direct assessment of the neuronal circuitries recruited by the visceral pain stimulus and, in the case of functional brain imaging is very similar to the monitoring of CRD responses in healthy and IBS human subjects. Unfortunately, in animals these brain mapping techniques require euthanasia and limit the assessment to specific time points.

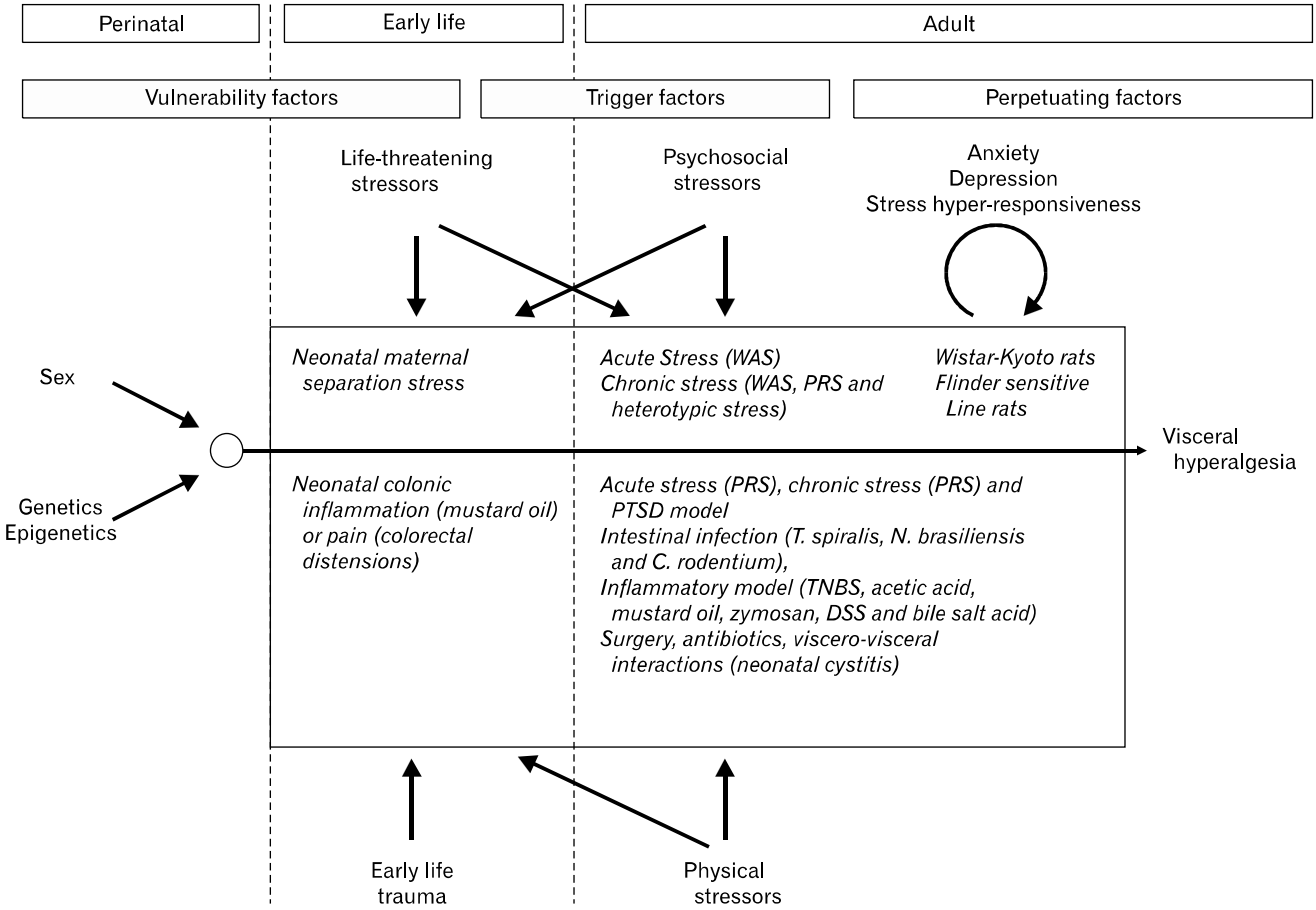

Figure 2. Animal models of stress-induced modulation of visceral sensitivity throughout the lifespan (Modified from Mayer et al[9]). Experimental stress models have been developed that target different periods throughout the lifespan of animals to assess the vulnerability, trigger and perpetuation influences of stress on visceral sensitivity. During early life, trauma due to maternal neglect (neonatal maternal separation stress) or injury (neonatal chronic colonic inflammation or pain) can enhance the susceptibility of individuals to develop altered visceral pain responses at adulthood. During adulthood, life-threatening stressors (post-traumatic stress disorder model), psychosocial stressors (acute and chronic stress) or physical stressors (intestinal infection or inflammation, antibiotic administration and surgery) have all clearly been established as triggering factors to the development of visceral hypersensitivity in rats and mice. Lastly, the use of specific strains of rodents known to exhibit various levels of anxiety, depression or stress hyper-responsiveness (Wistar-Kyoto and Flinders Sensitive Line) help mimic the influence of perpetuating factors on symptoms of visceral pain. WAS, water avoidance stress; PRS, partial restraint stress; PTSD, post-traumatic stress disorder; DSS, dextran sodium sulfate.

However, as more stringent brain imaging approaches are developed in rodents, they will open new venues to parallel human studies.[94]

Experimental Stress Models and Visceral Pain

By convention, stressors are categorized in exteroceptive (psychological or neurogenic) and interoceptive (physical or systemic) classes[95,96] and both have been used in animal models to investigate the relationship between stress and visceral pain.[97] Dual visceral pain responses: hyperalgesia and analgesia have been described in rodents exposed to exteroceptive stressors. Though only recently investigated, the analgesic response bears very relevant implications in the understanding of visceral pain-associated pathologies (detailed in section "Stress-induced visceral analgesia: how does it help us to model and understand visceral hypersensitivity?") In contrast, interoceptive stressors have been most consistently associated with the development of stress-induced hyperalgesia.

Stress modulates visceral pain in IBS patients as well as in healthy subjects,[9,98] therefore experimental animal models, involving exposure to various clinically relevant stressors have been developed to recapture features of IBS symptoms, of which hyperalgesia to sigmoid distensions is a hallmark.[99,100] Moreover clinical studies have stratified that the stress-related modulation of IBS symptoms[9] may be occurring through (1) permanent enhancement of stress responsiveness, (2) transient symptom exacerbation and/or (3) symptom perpetuation. Consequently existing experimental stress models target different periods throughout the lifespan of animals to assess the vulnerability, the trigger and perpetuating factors determining stress influence on visceral hypersensitivity (Fig. 2).

Stress in the Perinatal Period: Genetic/ Epigenetic Factors

Twin studies in IBS patients showed higher (but relatively low) concordance rates in monozygotic than dizygotic twins suggesting that although genetic factors are not dominant, they play a role in the occurrence of IBS.[101] There is also a growing literature reporting the association between functional genetic polymorphisms and IBS at the level of serotonin transporter gene (associated with diarrhea in female IBS patients), or α_2-adrenoreceptor gene (associated with constipation), and more recently, additional gene polymorphisms have been unraveled supporting the potential permissive role of genetics in IBS pathophysiology.[102-105] Of interest, it has been postulated that epigenetic factors related to heritable changes in gene expression that occur without alteration in gene sequence, determine the manner in which gene activity may be altered either transiently or permanently in response to environmental challenges.[106] Such epigenetic modifications could account for symptoms persistence, familial clustering and the transgenerational impact of IBS. However, experimental studies have not dwelled on strain differences in terms of stress responsiveness, anxiety and depression in rodents,[107-110] to assess and compare how genetic predisposition together with perinatal (maternal prenatal stress) or early life stressors (neonatal maternal separation stress) could affect visceral pain responses at adulthood in the context of epigenetic modifications. There is only one preliminary report suggesting that strain may determine the duration of the visceral hyperalgesia in response to chronic heterotypic stress (detailed in section "Stress in the adult period: trigger factors, Psychosocial stressors"). The influence of genes on the vulnerability of rodents to exhibit visceral hypersensitivity has however been assessed in relation with anxiety behavior at adulthood in rat strains with different anxiety/depression backgrounds[110] (detailed in section "Genetic models of anxiety & depression").

Stress in the Early-life Period: Vulnerability/ Trigger Factors

Early life events and childhood trauma by biopsychosocial factors (neglect, abuse, loss of caregiver or life threatening situation) enhance the vulnerability of individuals later in life to develop affective disorders (depression, anxiety and emotional distress) and put them at a greater risk for developing IBS.[12,99] In the context of epigenetic modifications, experimental studies showed that early developmental trauma decreases glucocorticoid receptor expression by hypermethylation of a key regulatory component and consequently affects the feedback regulation the HPA-axis with impact on the endocrine/behavioral adaptation and the susceptibility to stress-related disorders.[112] In addition, experimental studies indicate that the newborn's gut through stress-related changes in intestinal permeability may be exposed to a variety of factors resulting in mucosal inflammation and tissue irritation which could have long-term consequences at adulthood even though no longitudinal clinical studies exist showing that gut irritation in early life is a risk factor for IBS development at adulthood.[97] Moreover, postnatal microbial colonization has been also suggested as a potential factor programming the

HPA-axis response to stress in mice.[113]

An experimental model commonly used as a stress model to mimic early stress/childhood trauma is the neonatal maternal separation in rodents. This is achieved by isolating pups from the dam for 2-3 hours/day during the first 2 weeks after birth from postnatal day (PND) 1-2 to PND 14.[17,114-116] At adulthood, rats previously subjected to neonatal maternal separation exhibit visceral hypersensitivity to CRD under basal conditions which is further exacerbated by exposure to the acute psychological stressor in the form of water avoidance stress (WAS) consisting in placing rodents on a small platform surrounded by water for 1h.[117,118] Other models used repeated intermittent colonic irritation during the neonatal period (PND 8-21) either in the form of daily noxious CRD (60 mmHg-60 seconds distension twice separated by 30-minute period of rest) or by performing daily intracolonic injection of mustard oil (5%), increases pain behavior to CRD from postnatal week 5 up to postnatal week 12.[89,119] Likewise, an acute somatic injury (saline or carrageenan injections into the hind paw) performed during the critical period of postnatal development, ie, before PND 14, produces visceral analgesia to CRD in adult rats.[120]

Based on these studies and the extensive amount of evidence originating from somatic pain studies,[121,122] it appears that neonatal insults either acute or repeated, somatic vs visceral occurring during the development of the organism contribute to induce a state of visceral hypersensitivity in adulthood which may reflect long-term changes in visceral sensory processing.[120]

Stress in the Adult Period: Trigger Factors

Psychosocial stressors

Psychosocial stressors (eg, threat to social status, social esteem, respect and/or acceptance within a group; threat to self-worth) activate stress circuits within the emotional motor system and induce neuroendocrine response (CRF and cortisol) and autonomic response (norepinephrine and epinephrine) that result in the modulation of gut sensory, motor and immune function as well as intestinal permeability.[9] In experimental studies, the 2 main acute stressors that are prominently used in visceral pain studies are WAS for 1 hour and partial restraint stress for 2 hours, a stressor with stronger psychological component than WAS, which entails taping the forelimb of rats in order to prevent their movements.[123-125] Exposure of male Wistar rats to WAS for 1 hour leads to the development of a delayed visceral hyperalgesia to CRD, appearing 24 hours after the end of the stress,[126] while exposure to partial restraint stress, induces an immediate hyperalgesia to CRD in male[127] and female Wistar rats.[115]

However, in the context of clinical studies in which daily chronic stress predicts the intensity and severity of subsequent symptoms in IBS patients,[4-6,99,128,129] a variety of chronic stress models divided in 2 categories have been recently developed in adult rodents. The first category consists in exposing animals repeatedly (over a few days to weeks) but intermittently (once or twice per day) to 1 or different stressors, with the aim of mimicking the daily exposure to psychosocial stress that humans can encounter through their personal and professional interactions. The second category consists in continuous exposure to stressors as part of change in internal state, for instance inflammation, or external milieu, for instance single housing, or social crowding which mimics the effect of social milieu in humans or using genetic rodent strains that have constitutive stress hyper-reactivity (Wistar Kyoto, Flinders Sensitive Line). In particular, repeated intermittent exposure to WAS is one of the first "chronic" stress model to have been adapted to the study of visceral hypersensitivity[81] and is presently widely used.[88,130,131] Initial studies in which the visceral pain response was monitored using EMG recording that entails surgical implantation of electrodes, male Wistar rats exposed to 10 consecutive days of WAS for 1 hour daily developed visceral hypersensitivity to CRD lasting up to 30 days after the end of the last session of WAS.[81,130] In our laboratories however, we found that when naïve male and female Wistar rats were exposed to a similar WAS schedule and their VMR was monitored by intraluminal colonic solid-state manometry, a technique that does not require surgery, animals developed visceral analgesia to CRD.[132] Similar results have been obtained in C57BL/6 mice[88] and analgesic vs hyperalgesic responses were established to be dependent upon preconditions (surgery and single housing) associated with the method of recording of VMR (Fig. 1).[88,133] Therefore, the impact of repeated mild stress such as 1-hour exposure to WAS on visceral pain response to CRD is largely influenced by the basal state conditions of the animals before applying the repeated stressor (detailed in section "Stress-induced visceral analgesia: how does it help us to model and understand visceral hypersensitivity?" and reference[88]). Repeated exposure to unpredictable sound stress has also been recently shown to provide a model of delayed visceral hyperalgesia in male Sprague-Dawley rats.[134]

Because habituation can occur in response to repeated exposure to an homotypic stressor,[135,136] heterotypic stress models with different and alternating modalities to induce stress have been recently developed. However male Wistar rats exposed ran-

domly to a combination of cold restraint stress (45 minutes), WAS (1 hour) or forced swimming (20 minutes), 1 stressor per day for 9 consecutive days develop visceral hypersensitivity only at 8 hours but not at 24 hours or 7 days after the end of the last stressor.[137] Interestingly however, the same regimen of alternating stressors in a different strain of rats, Sprague-Dawley, led to a sustained visceral hypersensitivity lasting up to 2 weeks after the end of the stressor (S. Sarna and J. Winston, pers. comm.), suggesting that the strain and therefore genetic background of the animals, affects the visceral pain responses to repeated intermittent exposure to different stressors.

Life-threatening stressors

Retrospective clinical studies indicate that living through or seeing a traumatic event, such as war, environmental disasters, rape, physical abuse or a bad accident in adulthood can lead to post-traumatic stress disorder (PTSD).[138-144] There is evidence of increased prevalence of GI symptoms, in particular IBS in PTSD sufferers including war veterans.[138-142] Additionally, patients with IBS who have experienced traumatic events may be at higher risk for other co-morbid psychiatric disorders than IBS patients without a trauma history.[141]

In adult rats, treatment with a relatively short-lasting session of shocks or a social confrontation with a predator or aggressive conspecific animals induces long-lasting (weeks-months) conditioned fear-responses to trauma-related cues, and a generalized behavioral sensitization to novel stressful stimuli that persists or grows stronger over time.[145-148] Repetitive balloon distention of the distal colon causes increased cardiovascular 'pseudoaffective' reflexes in pre-shocked rats compared to controls, 2 weeks after a single session of foot shocks.[145-148] Of note, female rats appear to show a different pattern of sensitized behavioral responsiveness to the same challenge, possibly pointing to sex-related alterations in the neuronal substrates involved.[149]

Interoceptive stressors

In approximately 10% of patients with IBS, the onset of symptoms began with an intestinal infectious illness.[150] Bile salt malabsorption resulting from infectious damage with organisms such as *Salmonella and Campylobacter* within the terminal ileum and right colon may also underlie some forms of post-infectious IBS.[151] Inflammation, antibiotic treatments, bladder infection and surgery may also contribute to the symptoms in some patients. Below are described some experimental models of interoceptive stressors that have been used to mimic these clinical conditions.

Post-infectious irritable bowel syndrome model. Prospective studies have shown that 3% to 36% of enteric infections lead to persistent new IBS symptoms depending on the infecting organism. In addition, the co-existence of adverse psychological factors at time of infection is also an important determinant to the susceptibility to develop post-infectious IBS.[152] Other risk factors include female sex and some psychological characteristics such as anxiety, depression and somatization.[152] While viral gastroenteritis seems to have only short-term effects, bacterial enteritis and protozoan and helminth infestations are followed by prolonged post-infectious IBS.[152] The vast majority of human post-inflammatory hypersensitivity symptoms are observed after bacterial infection (*Campylobacter*, *Shigella*, *Salmonella* or *Escherichia coli* infections).

In preclinical models, long-lasting visceral hyperalgesia has been observed in mice after transient intestinal inflammation induced by *Trichinella spiralis* infestation[153,154] or in rats infested by *Nippostrongylus brasiliensis*.[155] Recently, however, it was found that male C57BL/6 mice infected with *Citrobacter rodentium*, an attaching-effacing murine enteropathogen similar in its mechanisms of infection to enteropathogenic *Escherichia coli*, do not spontaneously develop visceral hypersensitivity symptoms assessed by the increase in EMG response to CRD[156] unless exposed to a stressor (WAS, 1 hr/day for 9 days) during the time of infection (S. Vanner and N. Cenac, pers. comm.).

Post-inflammatory irritable bowel syndrome model. Despite some controversies on the origin of the symptoms,[157,158] "IBS-like" symptoms appear to be common in patients in remission from ulcerative colitis.[15,159] In rats, chemical irritants applied to the colon such as acetic acid,[160] mustard oil[161,162] and zymosan[163,164] evoke short-term hyperalgesia associated with transmural tissue damage/colonic inflammation. Intracolonic trinitrobenzene sulfonic acid induces a severe colonic transmural inflammation and visceral hypersensitivity that develops at 4-5 days with the disappearance of symptoms by 14 days.[165,166] Interestingly, in 24% of rats there is reoccurrence of visceral hyperalgesia 16 weeks after the induction of inflammation, while there is no evidence of microscopic inflammation in rat colonic tissues at this time point.[166,167] In a similar manner, daily intracolonic instillation of bile acid deoxycholic acid for 3 days induces a mild, transient colonic inflammation within 3 days of administration that resolves within 3 weeks in adult male Sprague-Dawley rats. In this model, a persistent visceral hyperalgesia starts after 1 week of bile acid administration which lasts up to 4 weeks.[168]

Mild non-specific colitis and acute dextran sodium sulfate (DSS, 5% in drinking water for 5 days)-induced colitis have been

associated with increased responsiveness to CRD on days 5 or 60 after the induction of colitis in male Balb/c mice while chronic colitis induced by DSS (3 cycles of 5% DSS for 5 days/cycle and 15 days of normal drinking water in between each cycle) has not.[167] These results are in contrast with another study showing that 4% DSS in drinking water for 5-7 days-induced colitis but failed to cause the development of visceral hypersensitivity in response to CRD in C57BL/6 or Balb/c mice when tested on days 5, 12, 16, 20, 30, 40 or 51 after the induction of colitis.[170] These disparate findings suggest that inflammation alone may not always lead to visceral hypersensitivity and that the type of inflammatory insult and severity determine whether this will result in the development of postinflammatory hypersensitivity. The interaction between colonic inflammation and the development of visceral pain has to be substantiated in future investigations.[166]

Antibiotics. Patients treated with antibiotics for non-GI complaints are 3 times more likely to report functional bowel symptoms. Antibiotic use disrupts the intestinal microbiota, fragilizes the host's intestinal homeostasis and integrity of intestinal defenses,[171] and has been associated with IBS.[172] In support of this hypothesis, administration to Balb/c mice of an oral combination of non-absorbable antibiotics (neomycin, bacitracin and pimaricin) which disturbed the commensal intestinal microflora results in visceral hypersensitivity to CRD in these animals.[173] Paradoxically, clinical studies support that specific antibiotics (rifaximin or neomycin) are an effective treatment option in non-constipated IBS patients, over a 3-month period[174,175] or even longer,[176] thereby confirming the role of dysbiosis in developing IBS symptoms.[177]

Surgery and somato-visceral convergence. Despite controversies, studies suggest that IBS is associated with an increased risk of abdominal and pelvic surgeries.[178] Surgical procedure as both a visceral and psychological stressor can initiate a series of events that either disturb GI function and interactions within the brain-gut axis and/or alter gut microbiota, which consequently may lead to generation of IBS symptoms.[179] Hind paw (plantar) incision or injection of low pH (4.0) sterile saline in the gastrocnemius muscle of adult male Sprague-Dawley rats induce a significant visceral hyperalgesia to CRD that lasts up to 2 weeks after the somatic injury occurred.[180,181] As a model of postsurgical pain, the plantar incision model is particularly relevant because surgical procedures are relatively common and possible visceral hypersensitivity may also thus be a relatively common postsurgical event.[179] The impact of somato-visceral convergence has to be considered in experimental models of visceral pain where animals are surgically equipped within the abdominal wall with EMG electrodes[84] (detailed in section "Stress-induced visceral analgesia: how does it help us to model and understand visceral hypersensitivity?).

Viscero-visceral interactions: neonatal cystitis. A significant overlap is observed between IBS and urinary symptoms, in particular those resulting from interstitial cystitis (IC).[182] Like IBS, IC predominantly affects female patients (90%) and shows a high comorbidity rate with psychological disorders. By analogy to IBS, an increased number of mast cells have been found in bladder biopsies in IC.[183] Recurrent urinary tract infections during childhood correlate with the development of chronic pelvic pain, a condition that often overlaps with IBS.[184] In an animal model of bowel-bladder cross-sensitization, acute bladder chemical irritation causes a significant decrease in colorectal sensory thresholds to CRD.[185] Very recently, the induction of neonatal cystitis in female Sprague-Dawley rats at PND 14 was shown to result in colonic hypersensitivity to CRD during adulthood,[186] supporting a potential key role for viscero-visceral convergence in IBS and comorbid disorders such as IC and chronic pelvic pain.[182]

Stress in the Adult Period: Perpetuating Factors

There is a strong overlap between IBS and psychiatric disorders, as established by the high percentage (54% to even 94%) of IBS patients meeting the criteria for at least 1 primary psychiatric disorder, most notably mood and anxiety disorders.[182] Although comorbid psychiatric disorders seem to be not directly connected with the occurrence of IBS, they strongly influence how the symptoms are experienced, the individual illness behavior, and ultimately the outcome. The influence of cognitive aspects as well as motivational and emotional components on the processing of sensory information is mediated by extensive neuro-anatomical network with a pivotal role of the insular and anterior cingulate cortices.[9,187,188] Autonomic dysfunction, in particular decreased parasympathetic activity and increased sympathetic outflow observed in psychiatric disorders as well as in IBS,[16,189,190] has been also suggested to have a relevant impact on the neurally mediated regulation of colonic sensory-motor and immune function.[191] The neuroimmune cross-talk involving the stress-induced changes in vagal nerve activity and/or sensitization of mast-cells seems to play a critical role in altering visceral sensitivity and intestinal barrier.[192]

Genetic models of anxiety and depression

In a comparative study using 3 strains of rats known to have

varying levels of baseline anxiety, the high-anxiety Wistar-Kyoto rats had increased VMR to CRD compared to low-anxiety Sprague-Dawley and Fisher-344 animals suggesting a direct link between anxiety and visceral hypersensitivity.[111] In addition, compared to low-anxiety strains of rats, the sensitivity of high-anxiety rats was highly exacerbated by peripheral sensitization of the colon with a small dose of acetic acid.[111] Of note, Wistar-Kyoto rats are also considered as a model of depression,[193,194] as are rats from the Flinders Sensitive Line which exhibit increased cholinergic sensitivity compared to control rats of the Flinders Resistant Line.[195,196] Similarly to Wistar-Kyoto rats, Flinders Sensitive Line rats exhibit increased VMR to CRD as well as a blunted corticosterone response to acute noise stress compared to Flinders Resistant Line, suggesting a link between depression, HPA axis dysfunction and visceral hyperalgesia.[197]

Genetic models of chronic stress

Genetic models that blocked chronically the stress pathways by deleting CRF_1 receptors showed a decrease in anxiety and colonic sensitivity to CRD.[198] Conversely, genetic models of chronic stress relying on the over-expression of CRF stress system in mice[199] are available and could be useful to study IBS-like manifestations, but the visceral sensitivity of these transgenic animals has not been assessed yet. However, as CRF over-expressing mice display phenotypes of Cushing's syndrome,[200] new promising genetic models with more selective conditional and/or region-targeted genetic manipulations including RNAi gene silencing technology to modify CRF-related genes are continuously developed.[201-206] These models will be suitable to explore specific stress circuitries in the context of targeted chronic CRF expression/deletion and the impact on visceral pain modulation which so far is lagging behind.

Stress-Induced Visceral Analgesia: How Does It Help Us to Model and Understand Visceral Hypersensitivity?

While extensively described in somatic pain field,[207] to date activation of descending inhibitory pathways in stress-related visceral responses has received less attention. Opioids have been implicated in descending inhibition of visceral sensitivity following an acute stress as evidenced by the fact that naloxone unmasked WAS-induced hyperalgesia to CRD in normal Long-Evans rats and exacerbated the pain response to CRD in maternally-separated rats.[117] In another study, a non-opioid, neurotensin-dependent visceral analgesic response was observed 6 hours after exposure to an acute session of WAS in Sprague-Dawley rats, with males exhibiting stronger analgesia than females as well as in wild-type but not in neurotensin knock-out mice.[208] In another experimental model, a daily short period (15 minutes) of separation from PND 2 to 14, decreased VMR to CRD performed immediately after WAS and prevented the development of hyperalgesia 24 hours after WAS in adult male Long-Evans rats.[209] These data suggest a potential upregulation of endogenous pain-modulatory systems by this short maternal separation stress.[209] Similar findings in adult rats have been recently reported, such that Wistar rats handled daily for 9 days develop visceral hypoalgesia in response to CRD that becomes significant 7 days after the last handling.[137]

These studies point to the type of stress itself contributing to the differential recruitment of those descending inhibitory pathways. However, importantly, we recently demonstrated that mice that had undergone surgery for the placement of EMG electrodes on abdominal wall and were subsequently single housed to avoid deterioration of implanted electrodes by cage-mate, developed visceral hyperalgesia in response to repeated WAS (1 hr/day, 10 days) while mice tested for visceral pain using the non-invasive solid-state intraluminal pressure recording and kept group housed developed a strong visceral analgesia under otherwise similar conditions of repeated intermittent WAS.[88] As mentioned before surgery *per se* is known to induce a long lasting visceral hyperalgesia and recent reports suggest that previous injury or exposure to opioids in male rats can switch stress influence on pain responses from analgesia to hyperalgesia.[210] Collectively these data demonstrate that the state of the animal tested (naïve vs exposed to surgery), its social environment (group housing vs single housing, cage enrichment or not), the handling performed by the investigator, the methods used to record VMRs (EMG requiring surgery and antibiotic post surgery vs manometry not requiring surgery/antibiotic), as well as the sex of animals can significantly affect the response to exteroceptive stressors. Therefore these preconditions should be carefully detailed in describing the experimental conditions and taken into consideration in the design, conduct and interpretations of the data when investigating the influence of stress on visceral sensitivity in experimental animals.

Based on recent clinical findings demonstrating that IBS patients have compromised engagement of the inhibitory descending pain modulation systems,[21,211,214] gaining a deeper understanding of the mechanisms involved in the expression of stress-induced visceral analgesia or lack thereof are promising

avenues to be explored and may lead to new therapeutic targets for IBS. Therefore the use of non-invasive methods of monitoring VMR that alleviates the surgical, antibiotic and housing impacts on repeated stress modulation of visceral pain represents a step forward to gain insight into the underlying mechanisms in particular the neural substrates and neurochemistry of stress-related analgesia as established in the somatic field.[207]

Sex Differences in Stress-Induced Alterations of Visceral Sensitivity

Women are more susceptible to stress-related disorders which is consistent with female predominance in IBS patients (women to men ratio about 2:1).[215,216] Sex differences in the stress response and stress-induced pain modulation have been documented in a number of human studies.[217] Clinical trials have also revealed important sex-related differences in therapeutic efficacy of some serotonergic drugs used in IBS treatment (eg, alosetron, 5-HT_3 receptor antagonist) suggesting a conceivable link between estrogens and serotonergic mechanisms in the modulation of stress-related visceral hypersensitivity.[218,219] Contrasting with this clinical evidence, most of the preclinical studies assessing stress-related alterations in visceral sensitivity have been conducted in male rodents.[208,220] However, the few studies performed in female indicate that sex hormones have a significant effect on visceral sensitivity in rodents.[220-224] Therefore, addressing the influence of sex and sex hormones in the modulation of visceral pain by stress appears critical to develop novel therapies relevant to sex difference in IBS.[216,225]

Mechanisms Involved in Stress-Induced Modulation of Visceral Pain

Maladaptive neuroplastic changes leading to persistent increased perception and responsiveness to noxious stimuli, or response to normally non-noxious stimuli are key for the expression of pathological pain (hyperalgesia and allodynia). Such neuroplastic changes can occur in primary afferent terminals (peripheral sensitization) but also in the spinal cord (central sensitization) and in the brain (supraspinal pain modulation) or in descending pathways that modulate spinal nociceptive transmission. Such alterations in the processing of sensory information are all considered as possible mechanisms of visceral hypersensitivity in IBS patients.[66,226]

Peripheral Sensitization: Corticotropin Releasing Factor System, Mast Cells, Gut Microbiota and Ion Channels

Several reports in both humans and rodents have well documented the key role played by the peripheral CRF signaling, via CRF_1 receptors, in the development and expression of visceral pain.[19,60,227-231] Stress and peripheral administration of CRF induce mast cells degranulation in the colon in experimental animals and humans,[232,233] which contributes to the development of visceral hypersensitivity (Fig. 1) via the release of several preformed or newly generated mediators[118,234-237] (histamine, tryptase, prostaglandin E_2, nerve growth factor) that can stimulate or sensitize sensory afferents[66,238] by activating a number of ion channels widely expressed in colonic afferents[239-242] such as N-methyl-D-aspartate receptor,[242] proteinase-activated receptor,[236] and transient receptor potential vanilloid 1[243-245] to name a few.

Stress can also disrupt the intestinal epithelial barrier thereby increasing the penetration of soluble factors (antigens) into the lamina propria, leading to nociceptors sensitization,[235,246] a phenomenon which appears as a prerequisite for the development of visceral hypersensitivity in both humans and rodents.[246-248] Alterations of epithelial permeability following stress involves the activation of the peripheral CRF system and may[249-253] or may not be dependent from mast cell activation[238,253] in a time-dependent manner. In addition to inducing a leaky epithelial barrier, stress can also change the composition of the intestinal and fecal microbiota of rodents.[254-256] This can in turn have significant impact on the host and affect behavior, visceral sensitivity and inflammatory susceptibility.[257-261]

Spinal Cord Plasticity and Glia Activation: Central Processing of Peripheral Pain Perception

Once peripheral sensitization has occurred, it activates the release of mediators in the spinal cord including growth factors[262,263] (nerve growth factor or brain-derived neurotrophic factor) and upregulates some key ion channels and receptors such as acid-sensing ion channels 1A and neurokinin 1 receptor[264-267] contributing to the phenomenon of spinal sensitization which has been associated with visceral hypersensitivity.

Very recently, spinal cord glia activation has been suggested as being another potential mechanism through which spinal sen-

sitization may occur in response to stress linked to the development and maintenance of visceral hypersensitivity.[268-270] Candidate molecules involved in glia activation signaling include neurotransmitters such as susbstance P or glutamate, but also purinergic agents, opioids, chemokines and glucocorticoids (for review see reference[268]). Glutamate uptake through spinal glutamate transporters is critical for maintaining normal sensory transmission under physiologic conditions.[271,272] A potential deficiency in glutamate reuptake by astrocytes associated with the activation of spinal cord glia[273] has been recently suggested to play a role in the spinal sensitization and the development of visceral hypersensitivity in rats.[274] Together, these data strongly support the concept that transmission of visceral nociceptive signals may be enhanced in various conditions of spinal microglia activation.[275]

Supraspinal Pain Modulation: A Fine-tuning between Pain Facilitation and Inhibition

Various supraspinal sites are involved in the modulation of visceral pain signals. Rectosigmoid distension in humans activates sensory (insula and somatosensory cortex), and limbic and paralimbic regions (including anterior cingulate cortex, amygdala and locus coeruleus).[276] Many of these brain regions were also found to be significantly activated by CRD in rats.[25-27,33,277]

The anterior cingulate cortex mediates key emotional-aversive aspects of pain and may also have a mnemonic role in which it allows transient storage of information during pain processing.[189,278] Wistar-Kyoto rats, high-anxiety rats exhibiting visceral hypersensitivity[111] have greater prefrontal cortex activation in response to CRD compared to Sprague-Dawley.[91] Another key limbic system structure that has been implicated in the affective component of pain is the central amygdala. It is involved in the processing of visceral information, attention, emotion and integrating the physical and psychological components of the stress response.[279] It has also been found to contribute to visceral hypersensitivity in rats.[280-283] Of relevance in the context of stress response, the CRF gene expression in the amygdaloid nucleus is upregulated in a mouse model of visceral pain and such a response is attenuated under conditions of anesthesia.[283,284] Likewise, the locus coeruleus is a well established target of stress that expresses CRF_1 receptors, receives CRF innervation from nearby Barrington nucleus and increases firing in response to CRD that is mediated by CRF_1 receptor activation as shown by the use of CRF receptor antagonists and the responsiveness of LC neurons to both CRD and to central injection of CRF.[53,285-290] Therefore these limbic and pontine sites are well positioned to coordinate gut-brain interaction with visceral information from the gut impacting on cortical and limbic activities under conditions of stress-CRF_1 signaling activation which may modulate the visceral pain responses.[60,76,291]

Thalamic relay nuclei have a key role in gating, filtering and processing sensory information en route to the cerebral cortex and are subject to similar activity-induced plasticity processes as the spinal cord.[292-294] Upregulation of CRF_1 receptor in the thalamus is associated with visceral hyperalgesia in the rat model of neonatal maternal separation stress.[275] Lastly, spinal visceral nociceptive reflexes are subject to facilitatory modulation from the rostroventral medulla, providing the basis for a mechanism by which visceral sensations can be enhanced from supraspinal sites[295,296] under stress conditions associated with development of visceral hyperalgesia.[297] Compromised engagement of descending pain inhibitory pathways as observed in maternally-stressed rats may also contribute to increase the visceral pain responses in those animals.[117]

Therapeutic Implications-Treatment Targeting Stress Reduction in Irritable Bowel Syndrome

The modulatory role of stress-related brain-gut interactions in the IBS pathophysiology, in particular neuroimmune modulation associated with psychological factors and emotional state[16,189] has been confirmed by the encouraging outcome of non-pharmacologic and pharmacologic treatment modalities aimed at reducing stress perception.[298-300] A broad range of evidence-based mind-body interventions including psychotherapy, cognitive behavioral therapy, hypnotherapy, relaxation exercises or mindfulness mediation has been shown to amend stress coping strategies, both at a cognitive level (catastrophic or self-defeating thoughts) and at a behavioral level (problem solving, especially interpersonal problems).[300,301] The symptomatic improvement appears to result from the modulation of stress response, the autonomic nervous system balance restoration, and changes in the brain activation pattern in response to visceral stimuli. In addition to psychological mind-body approaches, clinical trials confirm the effectiveness of centrally-targeted pharmacological interventions such as with antidepressants, and anxiolytics, or combination of drugs from both groups in the treatment of chronic pain disorders.[299,302,303] Many other pharmacological agents with anxiolytic and/or antidepressant properties, such as serotonergic and opioidergic agents, cannabinoid receptor 1 (CB_1) and somatosta-

tin receptors agonists, CRF_1, tachykinin and cholecystokinin receptors antagonists, have been recently shown to modulate stress-induced visceral hyperalgesia in animal models (for detailed review see reference[304]). Preliminary data suggest that anxiolytic activity of γ-aminobutyric acid-ergic agents (gabapentin) and α2δ ligand (pregabalin) may be also efficient in reducing central sensitization in hyperalgesia in clinical setting[305] as shown in experimental models.[306] New centrally acting agents providing analgesic effects include dextofisopam (2,3-benzodoazepine receptor modulator) and quetiapine (atypical antipsychotic agent).[307]

Recent developments showing the critical interdependence between the composition and stability of the microbiota and GI sensory-motor function indicate a novel approach to IBS treatment with a use of probiotics, prebiotics and antibiotics.[260,308] Specific modulation of the enteric microbiota in the context of neuroimmune interactions within the brain-gut axis opens a new promising strategy for stress-related disorders, particularly in the aspects of comorbidity in functional GI disorders such as IBS.[257]

However, some of the encouraging data from animal models concerning efficiency in alleviating stress-induced visceral hypersensitivity of such agents as CRF_1 receptor antagonist,[309] CB_1/CB_2 receptor antagonist[310] or somatostatin receptor agonist (octreotide),[311] are yet to be confirmed in clinical trials, especially with regard to global symptoms improvement and well-being. For example, CRF_1 receptor antagonists are being investigated in Phase II/III clinical trials for depression, anxiety and IBS.[42] In fact, a recent clinical trial confirmed CRF_1 receptor antagonist efficacy in an anxiety model in healthy participants (7.5% CO_2 model).[312] Some observed discrepancies between preclinical models and clinical trials may result from limited correlation between readout from animal studies being based on pseudoaffective reflex responses or unlearned behaviors and symptoms in IBS patients reflecting subjective pain experience highly modulated by cortical influences.[1] As discussed in this review, the methods used to monitor visceral sensitivity in rodents by inducing some bias in the observed responses could also potentially contribute to the lack of clinical translation of some drugs.

Amelioration of animal models of visceral pain, in their construct and face validity, particularly through the development of non-invasive methods to monitor visceral sensitivity together with a recently emerging algorithm of drug screening based on pharmacological brain imaging techniques opens promising venues in establishing an adequate approach in identifying effective treatment for IBS symptoms as well as IBS-related quality of life impairment.

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